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CAMPESTERYL BEHENATE, A CHEMICAL CHARACTER OF THE LIVERWORT GENUS *CALYPOGEIA*[*]

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Key Word Index—*Calypogeia integristipula*; *C. neesiana*; *C. sphagnicola*; Marchantiopsida; liverworts; chemotaxonomy; campesteryl behenate; phytosterol.

Abstract—The discovery of campesteryl behenate in all species of the *Calypogeia* genus so far studied suggests that it is a characteristic feature of this liverwort genus.

INTRODUCTION

As a contribution to the chemotaxonomy of the liverwort [1], we have studied the composition of three species of *Calypogeia*, namely *C. integristipula* Steph., *C. neesiana* (Mass. et Carest.) K. Muell. and *C. sphagnicola* (Mass. et Carest.) Warnst. et Loeske. Our knowledge of the chemistry of this genus originates from Huneck [2,3], who found 1,4-dimethylazulene, 4-methyl-1-methoxycarbonylazulene and 3,7-dimethyl-methoxycarbonylindene in *C. trichomanis* (L.) Corda and unidentified bluish compounds [4] in *C. goebelii* (Schifn.) Steph. In *C. trichomanis*, *C. muelleriana* (Schifn.) K. Muell. and *C. integristipula* Steph., the presence of campesteryl behenate was recorded [5]. In preliminary work, one of us [6] reported the same compound in *C. meylanii* Buch; but this plant is now more correctly known as *C. integristipula*; the content of *n*-alkanes, cerides and their fatty acids in the petrol extract of this plant was published later [7].

RESULTS AND DISCUSSION

All the species now studied yielded as the main and typical component of their petrol extracts, after chromatography on silica gel, the same identical crystalline compound (mp 96–98°; hexane). Spectral data showed the presence of a sterol esterified by an aliphatic acid. After saponification, the presence of docosanoid (behenic) acid, identified as its methyl ester, and of campesterol, was proved. In this sterol, originating from all the species studied, an admixture of *ca* 3% sitosterol was detected, using GC. By another identification using reduction by $LiAlH_4$, and following acetylation [8], docosanyl and campesteryl acetates were obtained.

Consequently, the most significant compound present in all three species is campesteryl behenate. The presence of this sterol ester in all 5 species (of about 8 species registered in Europe) so far analysed suggests it is characteristic for the genus *Calypogeia*.

EXPERIMENTAL

For CC, Si gel (30–60 μm prepared according to ref. [9] and deactivated with 10% of water) was used; prep. HPLC was performed on Separon Si VSK (Laboratorní přístroje Praha): 10 nm in a column 8 × 500 mm, solvent *n*-hexane + 0.5% isopropanol. GC analyses were carried out on a PYE Series 104/64, on 3% SE-30 on GasChrom P at 250° (for sterols) or 210° (acid methyl esters). For TLC, Si gel G Merck was used.

Plant material. C. integristipula was collected in September 1970 near Jestřebice in Central Bohemia, *C. neesiana* and *C. sphagnicola* in August 1976 near Hora sv. Šebestiána in the Ore Mountains. *Campesteryl behenate*—carefully selected liverworts were dried at room temp., ground in a ball-mill, and extrd with

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petrol (bp 40–60°); from 120 g *C. integristipula*, 1.3 g of the extract was obtained, from 57 g *C. neesiana*, 1.0 g, and from 54 g *C. sphagnicola*, 1.2 g. The extract of *C. integristipula* and *C. neesiana* was chromatographed on Si gel columns. Using petrol with 2% benzene as eluant, crystalline compounds (77 and/or 49 mg) mp 95–96° were isolated, identical according to their IR (KBr), MS and NMR data. Using prep. HPLC, 40 mg of the same compound were isolated from *C. sphagnicola*. All spectral data of the three mentioned products are in accordance with those published by Huneck [5].

Alkaline hydrolysis. The crystalline ester (34.1 mg) originally, for example, from *C. integristipula*, was hydrolysed under reflux in ethanolic KOH (68.2 mg in 6 ml) for 3 hr. After the usual work-up, campesterol (13.0 mg), mp 156–157°, $[\alpha]_D^{25}$ $-32.3°$, (c 0.14) identical with an authentic specimen, was isolated. In the GC analysis an admixture of about 3% sitosterol was observed, and it was identified by co-chromatography with an authentic specimen. The same findings were confirmed by MS analysis. The acid originating from the hydrolysis (12.0 mg, mp 80°) was identified as behenic acid as the methyl ester and compared by GC with an authentic specimen.

Reduction and acetylation [8]. Campesteryl behenate (20.6 mg) was reduced in EtOAc (6.2 ml) soln of $LiAlH_4$ (51 mg), and then acetylated with Ac_2O. After the usual work up campesterol acetate (7.5 mg, mp 136°) and docosanol acetate (6.4 mg, mp 60–63°, ref. [10] 64–65°) were identified.

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ELLAGIC ACID 4-*O*-RUTINOSIDE FROM PODS OF *PROSOPIS JULIFLORA*

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Key Word Index—*Prosopis juliflora*; Leguminosae; pods; ellagic acid 4-*O*-rutinoside.

Abstract—From the pods of *Prosopis juliflora* a new glycoside, ellagic acid 4-*O*-rutinoside, has been characterized.

In our previous communications [1, 2] we have reported the presence of ellagic acid 4-*O*-α-L-rhamnosylgentiobioside in the pods [1] and 3,3-di-*O*-methylellagic acid 4-*O*-α-L-rhamnopyranoside in the roots [2] of *Prosopis juliflora*. We now report the isolation and characterization of another ellagic acid glycoside (**1**) from the pods of the same plant.

Compound **1** was found to be a non-reducing glycoside, which gave a dark bluish green precipitate with ferric chloride and yellow colour with alkali indicating its phenolic nature. Preliminary diagnostic tests, including a positive Greissmeyer reaction, suggested that it was an ellagic acid derivative. Absorption maxima at 238 nm and strong IR peaks at 3440 (—OH) and 1725 cm^{-1} (lactone) were similar to other ellagic acid derivatives [1–5]. On acid hydrolysis it gave ellagic acid [mp, colour tests, solubility UV, IR and co-paper chromatography (co-PC)] rhamnose and D-glucose. The sugars were identified by co-PC with authentic samples and preparation of their corresponding osazone derivatives. Quantitative acid hydrolysis showed the presence of 2 mol reducing sugar/mol ellagic acid and was further substantiated by elemental analysis of the glycoside and its derivatives.

The glycoside **1** was unaffected by aqueous alkali, eliminating the possibility of its being a sugar ester involving bidentate ester linkages with hexahydroxydiphenic acid. A bathochromic shift of 47 nm with sodium ethylate indicated the presence of at least one free